Colicins and the Energetics of Cell Membranes

The active transport of selected substances into the cell needs energy. Colicins, antibiotics made by bacteria, can stop active transport and can therefore be used to study how it is achieved

by Salvador E. Luria

Beyond the study of genes and gene action no biological problem presents a greater challenge than unraveling the diverse and subtle functions of cell membranes. Early in the evolution of life the invention of membranes must have come in sequence and significance next to the invention of genes. The seclusion of genes within a resilient envelope made it possible for them to function in an artificial and protected environment. The cell had come into existence.

The structure and function of cell membranes are being studied in many laboratories around the world by many techniques. One of the central problems is to explain how certain substances are actively transported through the membrane, a process that requires a considerable investment of cellular energy, whereas other substances are rigorously excluded. As often happens in research, useful information about a refractory problem is obtained as a by-product of an investigation originally undertaken with other objectives. It was by such a roundabout route that my co-workers and I at the Massachusetts Institute of Technology gained some insight into the way the cell membrane mobilizes the energy needed for active transport in the course of studying a family of antibiotic substances named colicins.

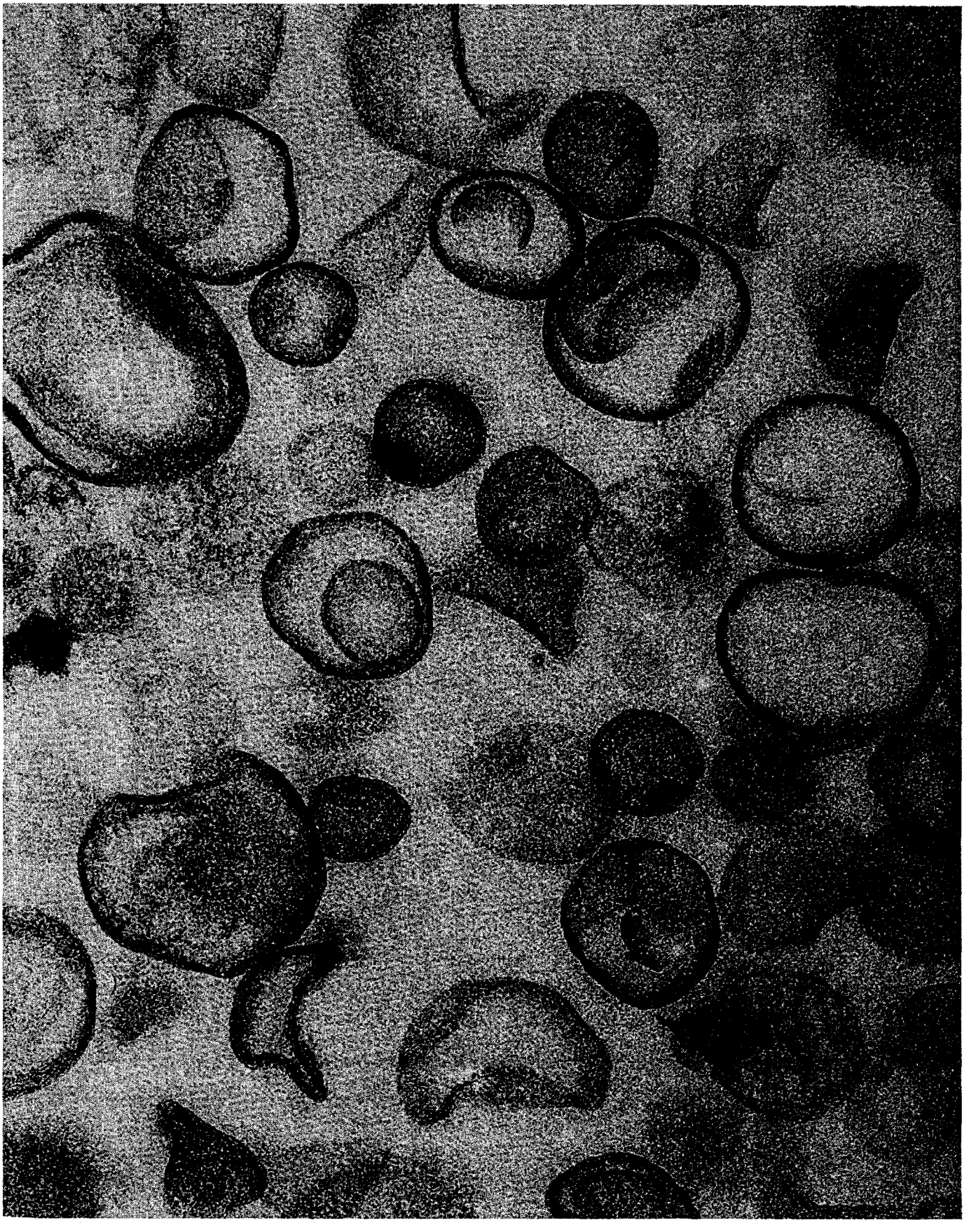
Colicins are a class of proteins manufactured by many bacteria of the *Escherichia coli* group. Each colicin is the product of one gene; the colicin genes are usually present in the special pieces of genetic material known as plasmids. Colicins kill the cells of bacterial strains related to the strains that make them but not the cells of unrelated strains. There are many different colicins, probably hundreds of them. No one has yet identified their biological role. They may be related to certain proteins of bacteriophages (the viruses that infect bacteria) or to proteins of bacterial membranes. This unsolved problem need not concern us here.

Before I describe what we have learned about active transport with the help of colicins let me summarize briefly how cell membranes are organized. Their main structural component is a double layer of phospholipid molecules. These molecules have water-attracting heads and water-repelling tails, so that in an aqueous environment they line up in a bilayer with their heads pointing toward the water and their tails pointing inward, away from the water [*see illustration on page 32*]. When a phospholipid is shaken with water, its molecules form a cloud of closed vesicles, or bubblelike arrays, because they seek a state of minimum free energy with all their heads in the water, either outside or inside the vesicle, and all their tails tucked away in the middle of the bilayer. That is why one can puncture a cell with a needle without causing it to burst or collapse: the molecules of the bilayer move toward one another to seal the hole.

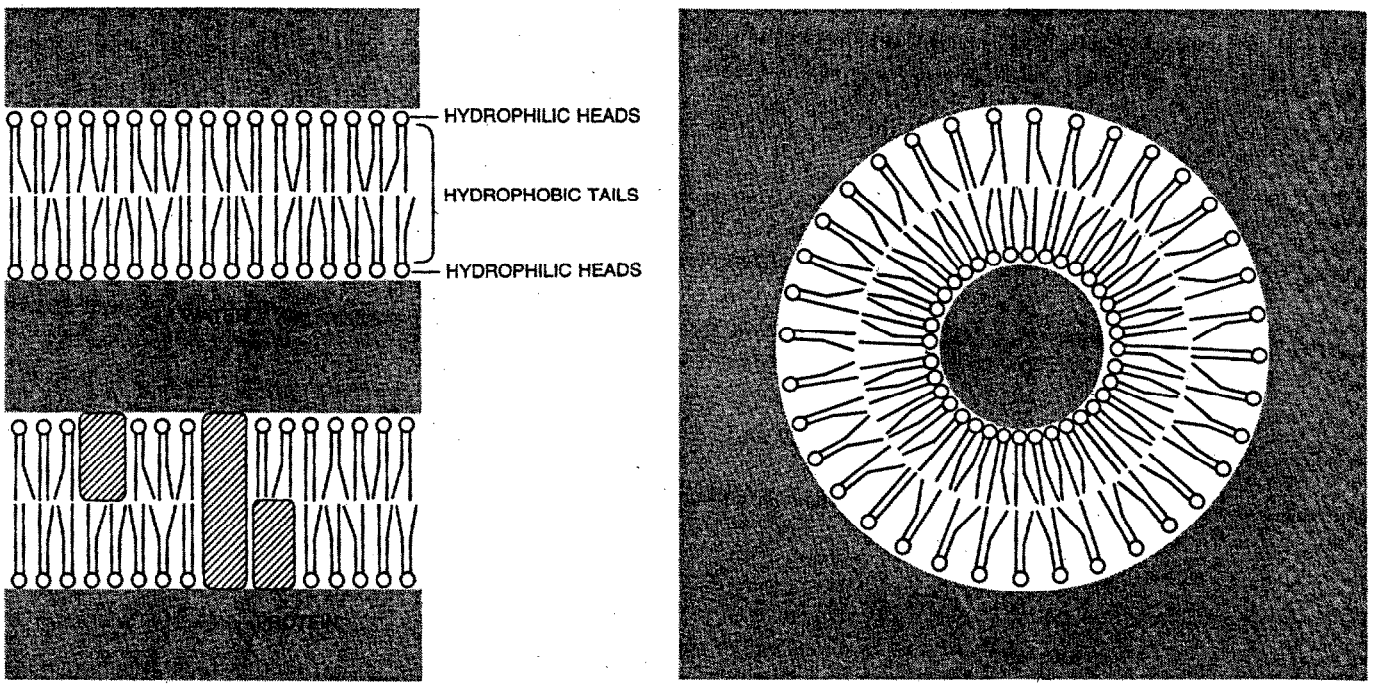
This self-sealing material is only the passive, mechanical part of a cell membrane. Substances are transported into a cell or out of it by protein molecules embedded in its outer membrane. As the cell grows, the components of the lipid bilayer are synthesized within the membrane by enzymes that also are embedded in the membrane. The membrane is a living, actively functioning structure. Moreover, it is not a static structure, a mosaic in which each tile is fixed where it happened to land or to be made. Cell membranes are quite fluid. Their components are remarkably free to move sideways within the two-dimensional layer. The functional activities of membranes may therefore involve changes both in the conformation of the membrane proteins and in the arrangement and association of these proteins.

That is the background to an array of membrane problems that intrigue the biochemist. How are the complex functions of the membrane coupled to and supplied with chemical energy? Consider a closed membrane vesicle, for example a bacterial cell, immersed in a dilute solution of potassium chloride. Although the lipid bilayer is impermeable to potassium ions, the membrane has protein channels that can transport such ions through the membrane. Actually live bacteria can concentrate potassium ions until the concentration inside the cell is as much as 1,000 times higher than the concentration outside. Such active transport, the accumulation of a substance against a concentration gradient, requires energy, just as the pumping of a liquid uphill against gravity requires energy. A water pump is actuated by an electric motor or some other source of mechanical energy. Cellular active transport is conducted by molecular pumps actuated by chemical energy. Poisons that interfere with cellular energy production inhibit active transport.

Active-transport systems are only one class of membrane functions that involve energy coupling. The activity of nerve cells and other excitable cells depends on the existence of electric membrane potentials generated by the expenditure of energy. Muscle contraction calls for the transfer of calcium ions in



MEMBRANE VESICLES, empty "bubbles" spontaneously reconstituted from the membranes of disrupted cells of the bacterium *Escherichia coli,* are shown in cross section in this electron micrograph. The two-layer structure of the cell membrane is clearly visible. The layers consist of molecules with water-attracting heads and water-repelling tails (*see illustration on next page*). Thus when fragments of cell membrane are shaken with water, the bilayer structure tends to seek a state of minimum free energy where the water-repelling tails are shielded from water. The minimum energy state is a bubble. Vesicles were prepared by H. Ronald Kaback of Roche Institute of Molecular Biology; micrograph was made by Vincent T. Marchesi of National Institute of Arthritis and Metabolic Diseases. The magnification is 40,000 diameters. At this magnification an *E. coli* cell would be 10 centimeters long.



TWO LAYERS OF CELL MEMBRANE consist of phospholipid molecules (*top left*). Each molecule has a hydrophilic (water-attracting) head and two hydrophobic (water-repelling) tails. In the membrane bilayer the tails face inward. The membrane is able to accommodate protein molecules of various shapes (*schematically indicated at bottom left*). In the presence of water the phospholipid bilayer forms a closed vesicle (*right*), a configuration that minimizes water-fat contact. Proportion of unsaturated fatty acids (*bent tails*) and temperature determine fluidity of bilayer and indirectly control the activity of the proteins in it or on it.

and out of membranous bags, a transfer that is energy-coupled. Transduction—the conversion of the energy of light or of mechanical stimuli into chemical and electrical signals in sensory cells—is another example of the coupling of the stimulating energy to changes in cellular membranes, leading in turn to the stimulation of sensory nerves.

How is this energy coupling achieved? In certain membrane-associated processes the chemical energy is provided by the splitting of the universal energy currency of living cells: adenosine triphosphate, or ATP. Transferring one phosphate group from one mole of ATP (475 grams) to water releases enough usable energy to melt 100 grams of ice. That amount of energy can be made available for pumping substances across a membrane. A much studied example of ATP's role in this process is found in the activation of muscle fibers. In order to remove calcium ions from muscle fibers to the membranous bags, where calcium is stored when the fibers relax, a particular protein molecule picks up two calcium ions and one molecule of ATP from outside the bag. One phosphate group is then transferred from ATP to the protein in such a way that when the phosphate comes off, it releases energy. Finally, the protein "flips" its calcium ions into the bag.

This neat picture, the outcome of years of sophisticated biochemical work in many laboratories, has one blank area in it. How does the energy released on the splitting of the phosphate group become available for the transfer of calcium ions? One can visualize, for example, a distortion of the carrier protein that enables it to rotate within the membrane and thereby deliver the ions into the bag. Alternatively the protein might be altered, possibly with the help of neighboring molecules, in such a way that a channel would be created through which calcium ions held by electrostatic forces could be led. Both in the study of enzyme action and in the study of membrane transport every biological process becomes a problem of protein structural chemistry: how protein molecules alter their configuration when they participate in chemical reactions.

The splitting of ATP into ADP (adenosine diphosphate) and phosphate is not the only source of chemical energy for pumping substances across membranes. As we know from the work of Saul Roseman and his colleagues at Johns Hopkins University, certain bacteria have developed a remarkable trick for taking in sugars such as glucose using a phosphate donor other than ATP. Of even greater significance, H. Ronald Kaback of the Roche Institute of Molecular Biology has discovered that in bacterial membranes the active transport of many substances—including potassium ions, amino acids and some sugars—is energized by the oxidation of various chemical substances without requiring the transfer of phosphate groups. The energizing mechanism is coupled more or less directly to the oxidation process, that is, to the transfer of electrons from a higher potential to a lower one, for example from lactic acid to oxygen. The transfer of electrons from any one of many organic substrates to oxygen is the major source of ATP in animal and plant cells. In bacteria the electron-transfer process can in addition provide energy for active transport directly, without going through ATP.

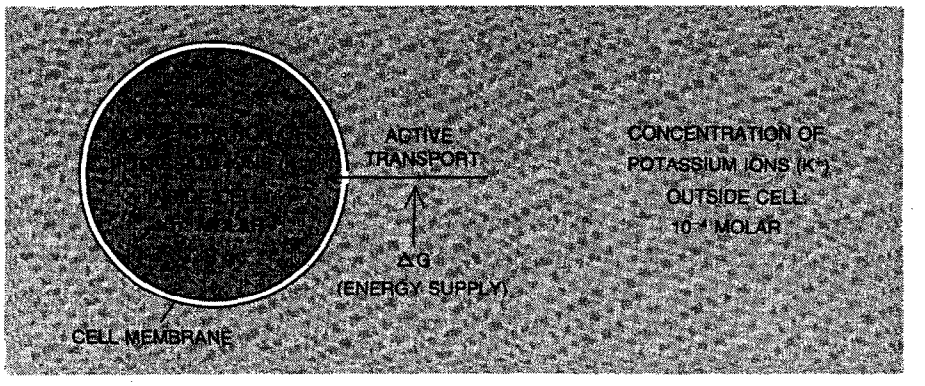
We are therefore led to visualize an energized state of the bacterial membrane that serves as storage for energy released by electron transfer. The energized state can be utilized to provide energy for active transport and also to generate ATP for cellular functions. Alternatively, by the reverse process, ATP made in the cell can be utilized to generate the energized membrane state.

A theoretical model of the energized state that is currently favored by many workers is the one proposed by Peter Mitchell of the Glynn Research Laboratories in England. In this model energy released by the transfer of electrons or the splitting of a phosphate group from

ATP would be stored in the form of a proton gradient, created by a mechanism that splits water so that protons (H^+ ions) accumulate on one side of the membrane and hydroxyl ions (OH^-) on the other [*see illustration on next page*]. The proton gradient contributes to the establishment across the membrane of an electric potential of about 200 millivolts, with the inside being negative. The energy stored in a proton gradient, as in an electrical condenser, could be used to pump substances into the cell in a variety of ways, for example by the association of one proton or more with a specific carrier molecule, which thereby becomes capable of transporting its substrate across the membrane. Such a model still leaves open the question of the actual molecular changes involved in the carrier's taking up the substrate, ferrying it across the membrane and releasing it into a region of higher concentration, such as the interior of a cell.

Against this background I shall now describe what we have learned about active transport from our work with colicins, which we began studying in 1963. Colicins were discovered some 50 years ago by the Belgian bacteriologist André Gratia and were later investigated by Pierre Fredericq in Belgium and by Masayasu Nomura in the U.S. They seem to fall into three classes, according to how they kill susceptible bacteria. For each class there is a specific biochemical target, that is, a cellular function that is blocked by colicin action. One class of colicins, designated *E2*, damages DNA. A second class, *E3*, damages the ribosomes, which are an essential component of the machinery for protein synthesis. Here we shall be concerned not with the *E2* and *E3* classes but with the third group, exemplified by colicin *E1* and colicin *K*, which are similar in the way they act and whose action is relevant to energy mechanisms in membranes.

When susceptible *Escherichia coli* cells take up colicin *E1* or colicin *K*, an entire series of things seem to go wrong. The synthesis of macromolecules—proteins, DNA, RNA and glycogen—stops almost immediately. This effect was observed some 20 years ago, and it suggested that the colicins damage some basic cellular mechanism. When I started to work on colicins in 1963, I was motivated by the possibility that these substances affected some master control mechanism of cellular syntheses. What I soon found was that the range of action of the *E1* and *K* colicins was even broader. The exposure of bacteria to colicin



$$\Delta G = \text{ENERGY OF CONCENTRATION}$$

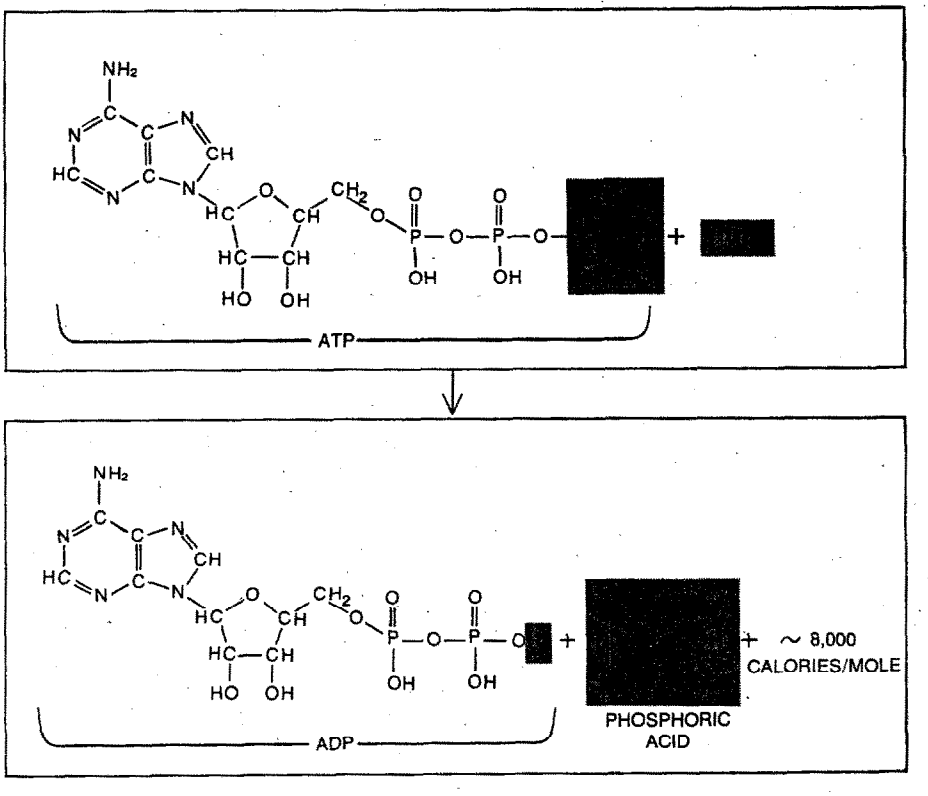
$$= 1{,}386 \log_{10} \times \frac{\text{CONCENTRATION INSIDE}}{\text{CONCENTRATION OUTSIDE}} \text{ CALORIES/MOLE}$$

$$\text{FOR : } \frac{\text{CONCENTRATION INSIDE}}{\text{CONCENTRATION OUTSIDE}} = 10^3$$

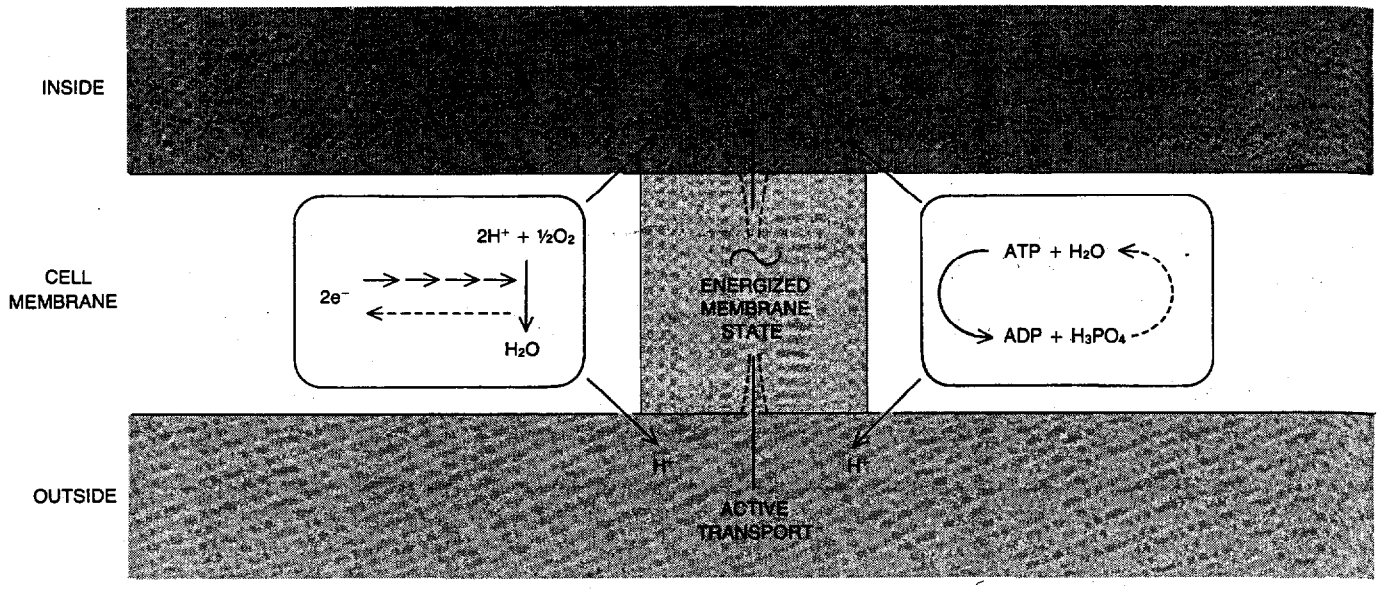
$$\Delta G = 3 \times 1{,}386 = 4{,}158 \text{ CALORIES/MOLE}$$

ENERGY IS REQUIRED to move any substance from a dilute region to a more concentrated one. Inside cells the concentration of many substances is hundreds or thousands of times higher than it is in the surrounding fluid. The cell is able to achieve such concentrations by active transport, which pumps selected substances through the membrane against a "head" that is higher inside the cell than outside. Free energy, ΔG, required can be calculated by equation. A mole of a substance is an amount equivalent to its molecular weight in grams. A major source of chemical energy available to the cell is shown below.

$$ATP + H_2O \rightarrow ADP + H_3PO_4 + \sim 8{,}000 \text{ CALORIES/MOLE}$$



CHEMICAL ENERGY is released by the cleaving of adenosine triphosphate (ATP), the ubiquitous energy currency of the cell. The released energy is made available when a phosphate group from ATP is transferred to water, yielding adenosine diphosphate (ADP) and phosphoric acid. Energy of ATP can be stored and used as it is needed for active transport.



ENERGIZED MEMBRANE STATE is generated either by the transfer of electrons from various substances to oxygen (*box at left*) or by the hydrolysis of ATP to form ADP and phosphoric acid (*right*). Both processes release energy. According to the chemiosmotic theory put forward by Peter Mitchell of the Glynn Research Laboratories, both processes are so oriented in membrane that they cause protons (H^+) to accumulate outside the membrane and hydroxyl ions (OH^-) to accumulate inside. In that way released energy is stored as a proton gradient. The resulting "protonmotive" force energizes the active-transport mechanism, indicated by the funnels. The protonmotive force can also energize reverse reactions of ATP hydrolysis and electron transfer (*broken arrows*).

E1 or colicin *K* promptly blocked active transport for the sugar lactose, various amino acids and potassium ions. The cells continued to take up and accumulate glucose, whose active transport, as I have mentioned, is energized by a special mechanism. This ability showed that the cells remained intact, even though they could not take up the other substances.

We soon found that what was defective was only the active phase of transport, the energy-demanding accumulation of various substances against a concentration gradient. The molecular mechanisms that exchange substrates across the cell membrane without the expenditure of energy were still functional. These results suggested that what the colicin did was to inhibit some phase of energy utilization.

The results were obtained in collaboration with my student Kay Fields, who is now at University College London. Since then a series of findings by our group (including at various times Gregory Brewer, David S. Feingold, Anton and Els Jetten, Joan Lusk, Charles Plate, Sohair Sabet and Joan L. Suit) has led us to our present view of the relation between colicins *E1* and *K* and the energy-coupling systems in the bacterial membrane.

It may be helpful to describe the nature of these experiments in somewhat more detail. Molecules of colicin attach themselves to a specific receptor on the bacterium. Sabet, working in Carl A. Schnaitman's laboratory at the University of Virginia School of Medicine before joining our group, isolated the receptors for some of the colicins and showed that they are protein molecules located in the bacterium's outer wall, which consists of lipopolysaccharide. The outer wall is a tough, comparatively inert envelope. The metabolically active membrane—the site of such processes as active transport, electron transfer, ATP production and the synthesis of various components of the envelope—is the cytoplasmic, or inner, membrane.

When the colicin is attached more or less firmly to a receptor, the bacterial cell is in what we call Stage I. The cell is killed when it enters Stage II. As long as the cell remains in Stage I it is still functionally normal and can be "rescued" by adding to the mixture of cells and colicin certain agents, such as the enzyme trypsin, that digest the colicin. The transition is defined by experiments in which one measures how many cells can be rescued by trypsin added at various times after the addition of colicin. The results are straightforward: The transition from Stage I (rescuable) to Stage II (killed) is a first-order, or "one hit," reaction, whose rate is directly proportional to the amount of colicin. The interpretation is also straightforward: What kills a cell is the action of one molecule of colicin. Increasing the amount of colicin only increases the probability of killing events per unit of time.

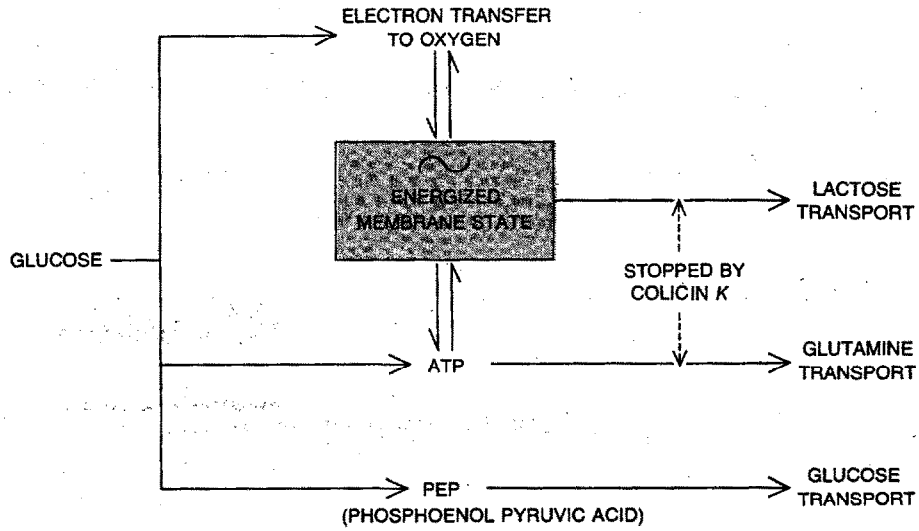
Before coming to the biochemistry of the killing process I should point out that the transition between Stage I and Stage II is itself interesting. In the first place, as Plate showed, its rate depends sharply on the physicochemical state of the lipids in the cytoplasmic membrane. If we make the membrane lipids less fluid, either by lowering the temperature or by forcing the cells to incorporate an unusual fatty acid in their lipids, the killing transition is greatly retarded. For colicin to kill a cell the cell membrane must be in a fluid state. This fact suggests either that the colicin must actually be transported through the lipid layer or that the reactions that follow the attachment of colicin require that the lipids be in a fluid state.

Even more interesting is the fact that the killing requires that the cytoplasmic membrane be energized. Any chemical treatment that interferes with the flow of energy in the membrane keeps the colicin-treated cells in Stage I, that is, it keeps them rescuable by trypsin. Why is membrane energy needed for the transition from Stage I to Stage II? I shall put off giving an interpretation until I have further described how the bacterial cells are killed by colicins.

I have mentioned that the killing of bacterial cells by colicins *E1* or *K* inhibits the functioning of various active-

transport systems. The inhibition is detected within a matter of seconds after the cells reach Stage II, when they can no longer be rescued by trypsin. Another event also takes place: the level of ATP in the cell is reduced between 30 and 50 percent, depending on the conditions of the experiment. Since ATP is the energy currency of the cell and many processes are likely to be sensitive to the amount of it that is present, it seemed possible that the lowering of the ATP level was the primary effect of colicin *K*.

We followed this appealing lead for quite a while, but it went nowhere. In the past year or so we have acquired a better understanding. We had been working with normal *E. coli* bacteria, and we decided for a number of reasons to try certain mutant strains that lack the enzyme ATPase (adenosine triphosphatase). This is the membrane enzyme that both catalyzes the synthesis of ATP, using the energy of electron transfer in the cytoplasmic membrane, and splits ATP to provide energy to the membrane. Bacteria without ATPase are, so to speak, schizoid with respect to energy: they can use glucose or other sugars to make ATP inside the cell and can also use any one of a variety of substances as a source of electrons to energize the membrane, but they are unable either to make ATP using the energy of electron transfer or to use ATP to supply energy to the membrane.

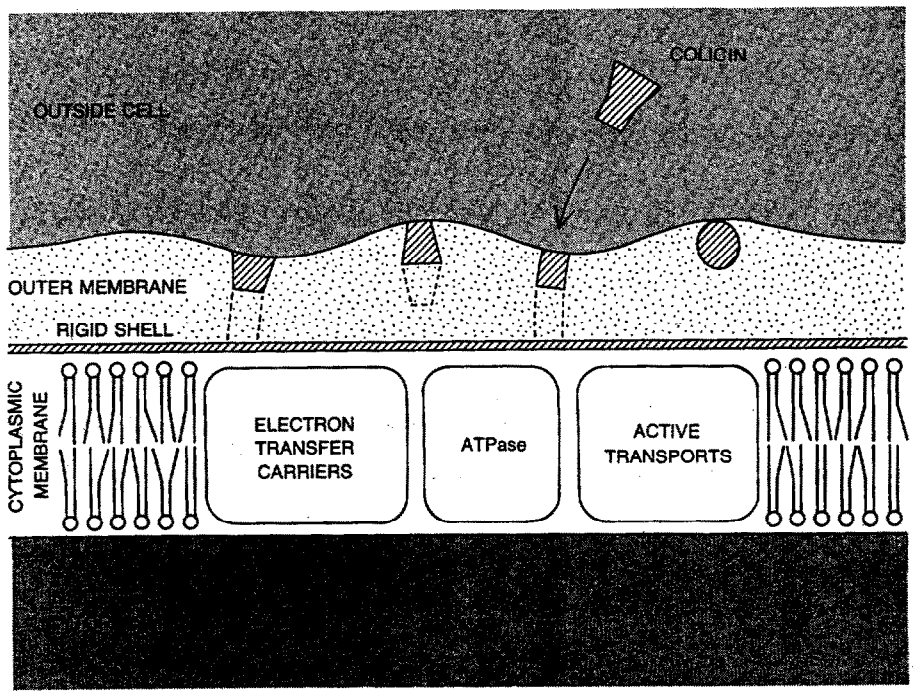


THREE ACTIVE-TRANSPORT SYSTEMS are available to *E. coli* cells that are supplied with glucose as a nutrient. The energized membrane state suffices for the active transport of lactose and many other substances, including the ions of potassium and magnesium. The transport of a second class of substances, including glutamine, requires ATP as such. The transport of a third class of substances, such as glucose, requires phosphoenolpyruvic acid (PEP). Bacterial product colicin *K* inhibits first two transport systems but not the third.

The action of colicin *K* on the ATPase-less bacteria turned out to be significantly different from the action on normal bacteria, and the differences were illuminating. The ATP levels went up instead of down and the synthesis of proteins and nucleic acids continued instead of stopping, but the various kinds of active transport were just as completely inhibited as they were in normal bacteria. The blocking of protein synthesis and nucleic acid synthesis, which we had thought was the main cause of cell death, was actually only a side effect of colicin action, probably reflecting the decreased ATP levels. The key action of colicin, we finally discovered, was on the utilization of membrane energy.

Why should colicin make the ATP levels go down in normal bacteria and up in ATPase-less mutants? We believe colicin *K*, by de-energizing the membrane, causes a drain of energy. If the ATPase enzyme is present, it wastes the cellular ATP in a vain attempt to re-energize the membrane. The situation can be compared to one where a pump exhausts a water reservoir in an attempt to replenish a leaky water tank. In the mutant bacteria that lack ATPase the leakage of energy due to colicin does not occur; the ATP level within the cell actually rises and the synthesis of macromolecules can continue. Such cells are dead only because a monkey wrench has been thrown into the machinery that supplies membrane energy.

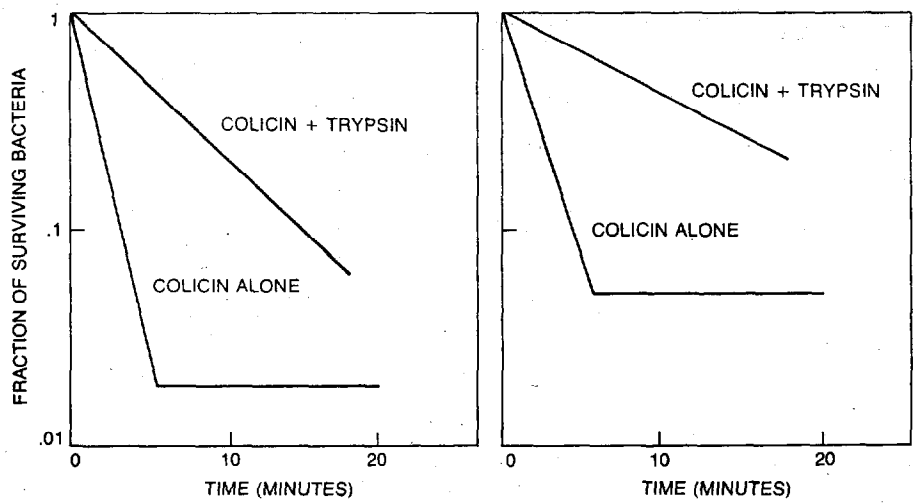
That interpretation has been supported by an elegant experiment performed by Lusk, who is now at Brown University. Reasoning that potassium and magnesium ions are probably the essential



BACTERIAL ENVELOPE has three major components: an inner cytoplasmic membrane, which has a backbone consisting of phospholipid bilayer; a rigid shell of peptidoglycan, which gives the cell its specific shape, and an outer membrane, which contains specific receptors (*hatched*) for various substances such as bacterial viruses and colicins. In some way the receptors convey their corresponding substrates to the cytoplasmic membrane. Embedded in the cytoplasmic membrane are protein molecules that serve a variety of functions. Arrangement that enables them to operate in production-line fashion is not known.

substances the cell must pump in from the outside, she treated the mutant bacteria that lacked ATPase with colicin *K* and then placed them in a culture medium containing high concentrations of potassium and magnesium. She found that in this medium the mutant cells, which otherwise would have been killed, survived, divided and gave rise to colonies of healthy, but still mutant, descendants!

How does the colicin specifically affect the coupling of membrane energy and active transport? There are a number of substances, such as cyanide, that de-energize membranes by blocking the transfer of electrons. Colicin *K* does not do that: in colicin-treated bacteria the rate of flow of electrons from various substrates to oxygen is normal. Other substances, known as uncouplers, de-energize membrane functions by blocking the conversion into usable membrane energy of the energy from electron transfer. These substances facilitate the passage of protons through membranes and abolish the proton gradient that provides energy storage in membranes. Colicin *K* does not act in that way either: it does not abolish the proton gradient. Moreover, it inhibits some transport systems that are insensitive to uncouplers. Brewer, experimenting with an indicator substance whose fluorescence reflects transmembrane electric potentials, found that what colicin *K* does is to lower the potential. That result would be expected from any action interfering with the creation or maintenance of the energized state, either by ATP or by electron transfer.



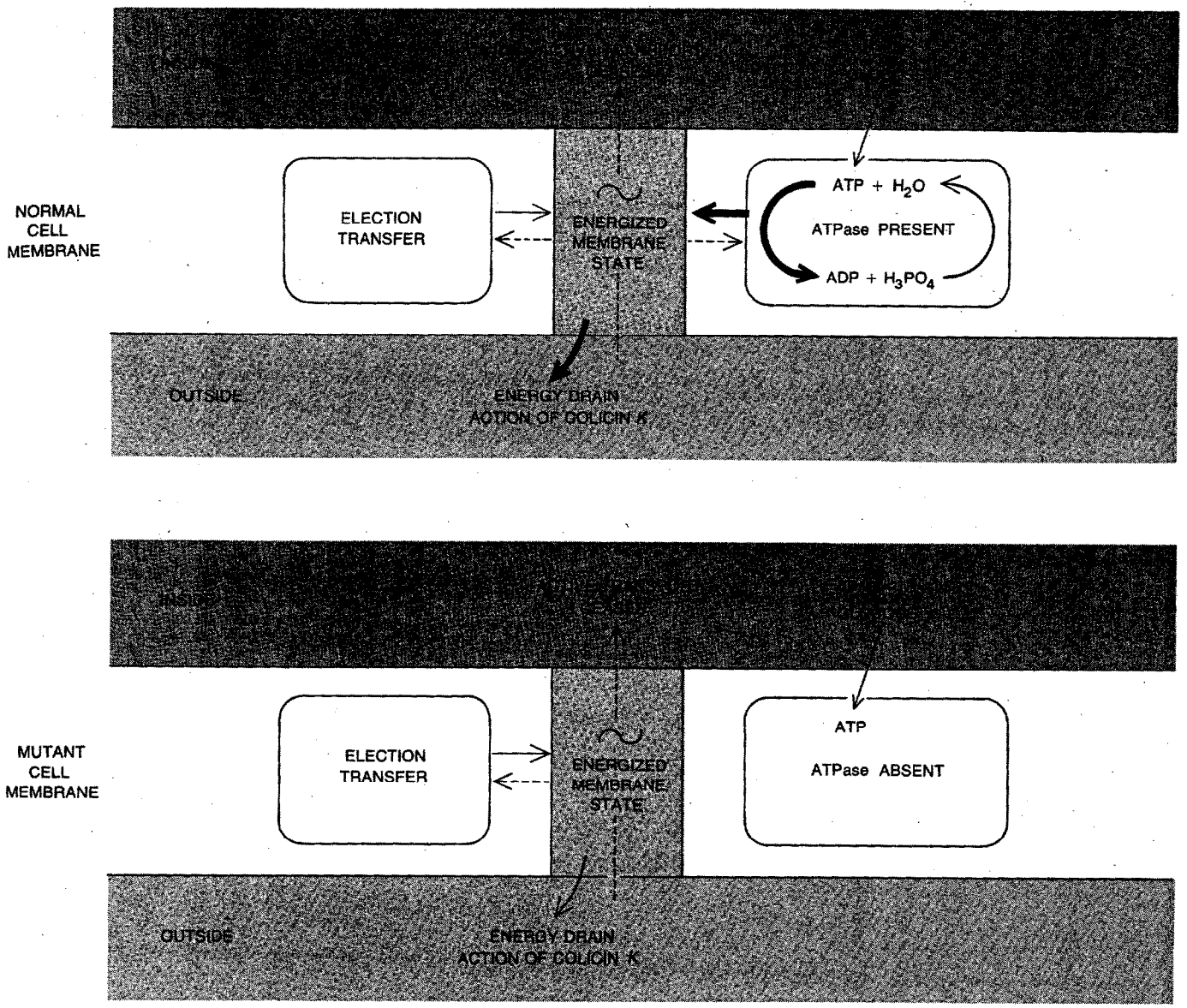
LETHALITY OF COLICIN *K* to *E. coli* is determined by comparing survival rates of cells exposed to different concentrations of colicin and seeing how many can be "rescued" by adding trypsin, an enzyme that destroys colicin molecules. Bacterial cells are mixed with colicin *K* at a concentration of one microgram per milliliter (*left*) or .5 microgram per milliliter (*right*). After five minutes both mixtures are diluted. Samples of the dilute mixture are removed at intervals and are assayed directly for their ability to produce colonies on agar (*black curves*). The surviving colonies represent bacteria that did not receive a killing dose of colicin. The colored curves show the fraction of bacterial cells that can be rescued by mixing them with large amounts of trypsin. The colored curves therefore indicate the proportions of cells that had not yet been killed at the moment trypsin was added. The fact that colored curves are straight lines whose slopes are proportional to amounts of colicin indicates that action of one colicin molecule is enough to kill one *E. coli* bacterium.

	ATPase PRESENT	ATPase ABSENT
PROTEIN SYNTHESIS	STOPS	CONTINUES
DNA AND RNA SYNTHESIS	STOPS	CONTINUES
ATP LEVELS	DECREASE	INCREASE
MOTILITY	STOPS	STOPS
ACTIVE TRANSPORT OF GLUCOSE	CONTINUES	CONTINUES
ACTIVE TRANSPORT OF GLUTAMINE, LACTOSE, ETC.	STOPS	STOPS

EFFECTS OF COLICIN *K* ON BACTERIA differ markedly depending on whether the *E. coli* cells are normal cells or are mutant cells that happen to lack adenosine triphosphatase (ATPase). ATPase is an enzyme that can catalyze either the synthesis of ATP, using the energy of electron flow in the cytoplasmic membrane, or the reverse reaction: the hydrolysis of ATP to provide energy to the cytoplasmic membrane. When colicin *K* is added to cells that contain ATPase, the synthesis of protein, DNA and RNA stops and the level of the ATP inside the cell falls sharply. When colicin *K* is added to mutant cells, it has little effect on synthesis of proteins, DNA and RNA; level of ATP in cells actually rises.

One way of looking at the coupling of membrane energy to active transport or to the synthesis of ATP is to postulate that energy is funneled inside the membrane to various systems, not directly by changes in the electric charge and conformation of ATPase or of the transporter molecules but through the agency of one protein or more whose deformation is somehow conveyed to the actual transporters. Colicin *K* might act on some of the intermediate steps and thereby inhibit a number of different transport systems. This hypothesis predicts that one should be able to find mutant bacteria in which the postulated intermediate steps are altered. Indeed, Plate has succeeded in isolating some *E. coli* mutants that have become resistant to colicin *K* and have also become altered in one way or another in the coupling of membrane energy to various transport functions. At least one such mutant mimics the action of colicin *K* and may alter the primary target on which the colicin acts. These findings support the idea that energy coupling involves a number of different steps and opens the way to using the method of genetic analysis for the study of membrane energy mechanisms. The program is now to isolate a series of mutants each of which is defective in one or another of the components of the energy-coupling system and to identify the corresponding biochemical functions.

As I have indicated, one property common to colicins of different classes is that they can kill only bacteria whose membrane is energized. In other words, the transition between Stage I and Stage II requires membrane energy. Anton Jetten, who has studied the phenomenon in detail, has found that in the absence of oxygen the ATPase-deficient bacteria take up colicin but are not affected by it, even though the bacteria are well supplied with ATP. In the absence of oxy-



DIFFERENCE IN COLICIN-K ACTION on normal *E. coli* cells and on mutant cells that lack ATPase throws light on how energy is supplied to produce the energized membrane state that is needed for active transport. In normal cells (*top*) colicin *K* stops active transport by draining the energized state of the membrane. In a fruitless attempt to maintain the energized state ATP is hydrolyzed to ADP inside the membrane and wasted. As a result the level of ATP inside the cell falls. In a mutant bacterium that lacks ATPase (*bottom*) active transport also stops after exposure to colicin *K*, because of drainage of the energized membrane state, but there is no hydrolysis of ATP inside the membrane and hence no wastage. Inside cell ATP continues to be made from glucose. Level of ATP actually rises, probably because damaged cell uses less of the substance for growth and general metabolic processes.

gen the membrane is not energized. As soon as oxygen is restored the bacteria start dying.

While we were puzzling over the meaning of this finding, a discovery from Kaback's laboratory provided a suggestive analogy. Kaback and his colleagues were studying in *E. coli* the chemical interaction of a certain type of sugar and its transporter molecules in the membrane. They found that there was no interaction unless the membrane was energized, as though energy was needed for the transporter molecules to come in contact with the sugar molecules. The requirements for the chemical interaction were identical with those for colicins in the transition between Stage I and Stage II.

It is tempting to propose a bold generalization and suggest that the energizing process in the membrane causes the various proteins that act as transporters or receptors to become accessible to their respective substrates. Changes in membrane potential, for example, could cause conformational changes in protein molecules to make them stick out of the membrane or pull back into it. Such changes might also be involved in the functioning of ion channels whose opening and closing are the critical events in the excitable membranes of nerve and muscle.

The accessibility of receptors to substrate cannot, of course, be the whole story in the utilization of membrane energy. Active transport, which involves not just the passage of a relatively small number of ions across a membrane but a massive accumulation of substrates against concentration gradients, must also call for the expenditure of energy for the release of the substrate within the cell. This too may be accomplished by conformational changes in membrane proteins. The unscrambling of the molecular events that give cellular membranes their functional properties promises to be an arduous but nonetheless fascinating task.